IN THE SPECIFICATION

Page 1, replace the paragraph after the title with the following:

This application is a divisional of Application No. 09/916,201, filed July 27, 2001, now abandoned, which is a continuation-in-part of Application No. 09/467,893, filed December 21, 1999, now abandoned, which claims benefit of Provisional Application No. 60/113,783, filed December 23, 1998, the entire contents of which are hereby incorporated by reference in this application.

Page 1, replace the third paragraph starting at line 18 with the following:

Using an assay which detects release of IFN-γ from T cells, the inventors have shown that CD8 T cells specific for tuberculosis antigens exist during tuberculosis infection and have identified particular CD8 T cell epitopes from ESAT-6 which are ~~recognised~~ recognized. Such epitopes may be used to detect anti-mycobacterial CD8 T cells. The inventors have also shown the presence of CD8 T cells specific for tuberculosis antigens in healthy contacts suggesting that such cells may be protective against tuberculosis.

Page 1, replace the fourth paragraph starting at line 25 with the following:

Accordingly the invention provides a method of detecting an anti-mycobacterial CD8 T cell response comprising contacting a population of CD8 T cells of an individual with one or more peptides selected from the peptides ~~represented by~~ of SEQ ID NOS: 3, 4, 7, 8, 9, 10, 11 [[or]] and 12, and, optionally, one or two further peptides ~~represented by~~ of SEQ ID NOS: 1 and/or 2, wherein one or more of said peptides may be substituted by an analogue which binds a T cell receptor that ~~recognises~~ recognizes the ~~corresponding substituted~~ peptide, and determining whether CD8 T cells of the CD8 T cell population recognize the peptide(s).

Page 4, replace the fourth paragraph starting at line 19 with the following:

The epitope used may be selected from the minimal epitopes represented by or present in any one of SEQ ID NOS: [[s]] 1 to 12.

Page 4, replace the fifth paragraph starting at line 23 with the following:

The CD8 T cells which ~~recognise~~ recognize the peptide in the detection method have generally been ~~pre-sensitised~~ presensitized *in vivo* to antigen from a vaccine or from a mycobacterium infection. These antigen-experienced T cells are generally present in the peripheral blood of a host which has been exposed to the antigen at a frequency of 1 to 10^6 to 1 in 10^3 peripheral blood mononuclear cells (PBMCs).

Page 4, replace the seventh paragraph starting at line 31 with the following:

Determination of whether the T cells ~~recognise~~ recognize the peptide is generally done by detecting a change in the state of the T cells in the presence of the peptide or determining whether the T cells bind the peptide. The change in state is generally caused by antigen specific functional activity of the T cell after the T cell receptor binds the peptide. Generally when binding the T cell receptor the peptide is bound to an MHC class I molecule, which may be present on the surface of a PBMC or an antigen presenting cell (APC).

Page 5, replace the second paragraph starting at line 15 with the following:

Typically the specific binding agent is ~~immobilised~~ immobilized on a solid support. After the substance is allowed to bind the solid support can optionally be washed to remove material which is not specifically bound to the agent. The agent/substance complex may be detected by using a second binding agent which will bind the complex. Typically the second agent binds the substance at a site which is different from the site which binds the first agent. The second agent is preferably an antibody and is labelled directly or indirectly by a detectable label.

Page 6, replace the fifth paragraph starting at line 29 with the following:

The APC used in the method may be any cell which has MHC class I molecules on its surface. It may or may not be a ~~specialised~~ specialized antigen presenting cell, such as a B cell, dendritic cell or macrophage. The APC used in the method may be from the same host as the T cell or from a different host. The APC may be a naturally occurring APC or an artificial APC. The APC is capable of presenting the peptide to a T cell. It is typically separated from the same sample as the T cell and is typically co-purified with the T cell. Thus the APC may be present in MCs or PBMCs. The APC is typically a freshly isolated *ex vivo* cell or a cultured cell. It may be in the form of a cell line, such as a short term or ~~immortalised~~ immortalized cell line. ~~The APC may express empty MHC class I molecules on its surface~~.

Page 7, replace the first paragraph starting at line 8 with the following:

Typically in the method the T cells derived from the sample can be placed into an assay with all the peptides (i.e. a pool of the peptides) which it is intended to test [[(]]the relevant panel[[)]] or the T cells can be divided and placed into separate assays each of which contain one or more of the peptides. In the *in vivo* embodiment of the detection method the relevant peptide(s) will of course be administered to the host. Typically one or more, or all, of the peptides ~~represented by~~ of SEQ ID ~~NO's~~ NOS: 3, 4, 8, 9 and 10 are also used in the method. In another embodiment only the peptides ~~represented by~~ of SEQ ID ~~NO's~~ NOS: 1, 2, 3, 4, 8, 9, 10 and one of 11 or 12 are used in the method.

Page 7, replace the third paragraph starting at line 20 with the following:

In one embodiment peptide *per se* is added directly to an assay comprising T cells and APCs. As discussed above the T cells and APCs in such an assay could be in the form of MCs. When peptides which can be ~~recognised~~ recognized by the T cell without the need for presentation by APCs are used then APCs are not required. Analogues which mimic the original peptide bound to a MHC molecule are an example of such a peptide.

Page 9, replace the sixth paragraph starting at line 31 with the following:

The sequence of the epitope may be any of the sequences ~~represented by~~ of SEQ ID NOS: 1, 2, 3, 4, 8, 9, 10, 11 [[or]] and 12 or the sequence of an epitope present SEQ ID NO: 7 (other than the SEQ ID NO:10 sequence).

Page 11, replace the second paragraph starting at line 6 with the following:

The vaccination may be based on the specific epitopes discussed above which are, ~~represented by~~ or contained in, SEQ ID [[NO's]] NOS: 1 to 12. Thus the invention also provides a method of vaccination which leads to a CD8 T cell response, the T cells of which are specific for a CD8 epitope which is ~~represented by~~ SEQ ID [[NO's]] NO: 1, 2, 3, 4, 8, 9, 10, 11 or 12, or which is present in the sequence ~~represented by~~ of SEQ ID NO:7, comprising administering (i) a CD8 epitope which is ~~represented by~~ SEQ ID [[NO's]] NO: 1, 2, 3 or 4, or which is present in the sequences ~~represented by~~ of SEQ ID [[NO's]] NO: 5, 6 or 7, (ii) an analogue of the epitope which is capable of inhibiting the binding of the epitope to a T cell receptor, (iii) a precursor of (i) or (ii) which is capable of being processed to provide (i) or (ii) excluding ESAT-6 or fragments of ESAT-6, or (iv) a polynucleotide which is capable of being expressed to provide (i), (ii) or (iii). (iii) may be the ~~peptides represented by~~ peptide of SEQ ID NO: 5, 6 or 7.

Page 11, replace the third paragraph starting at line 18 with the following:

The invention also provides these particular epitopes, analogues, precursors and polynucleotides represented by (i), (ii), (iii) and (iv), which may be in a ~~pharmaceutically~~ pharmaceutical composition in association with a pharmaceutically acceptable carrier or diluent.

Page 11, replace the fifth paragraph starting at line 30 with the following:

The analogue of a peptide can bind to a T cell receptor which ~~recognises~~ recognizes the original peptide. Therefore generally when the analogue is added to T cells in the presence of the original peptide, typically also in the presence of a presenting cell, the analogue inhibits the recognition of the original peptide. The binding of the analogue to the said T cell receptors can be tested by standard techniques. Such

T cell receptors can be isolated from T cells which have been shown to ~~recognise~~ recognize the peptide (e.g. using the method of the invention). Optionally such T cells may be sorted based on their ability to ~~recognise~~ recognize the original peptide, for example using a FACS technique. Demonstration of the binding of the analogue to the T cell receptors can then shown by determining whether the T cell receptors inhibit the binding of the analogue to a substance that binds the analogue, e.g. an antibody to the analogue. Typically the analogue is bound in an MHC class I molecule in such an inhibition of binding assay.

Page 14, replace the third paragraph starting at line 12 with the following:

The analogue may be ~~immobilised~~ immobilized on a solid support, particularly an analogue which mimics peptide bound to a MHC molecule.

Page 14, replace the fourth paragraph starting at line 14 with the following:

The analogue is typically designed by computational means and then ~~synthesised~~ synthesized using methods known in the art. Alternatively the analogue can be selected from a library of compounds. The library may be a combinatorial library or a display library, such as a phage display library. The library of compounds may be expressed in the display library in the form of being bound to a MHC class I molecule, such as the MHC molecule which the original peptide binds. Analogues are generally selected from the library based on their ability to mimic the binding characteristics of the original peptides. Thus they may be selected based on ability to bind a T cell receptor or antibody which ~~recognises~~ recognizes the original peptide.

Page 14, replace the sixth paragraph starting at line 29 with the following:

Thus the means may allow detection of a substance secreted by the T cells after recognition. The kit may thus additionally include a specific binding agent for the substance, such as an antibody. The agent is typically specific for IFN-γ. The agent is typically ~~immobilised~~ immobilized on a solid support This means that after binding the agent the substance will remain in the vicinity of the T cell which secreted it. Thus

'spots' of substance/agent complex are formed on the support, each spot representing a T cell which is secreting the substance. Quantifying the spots, and typically comparing against a control, allows determination of recognition of the peptide.

Page 15, replace the second paragraph starting at line 12 with the following:

The ~~immobilised~~ immobilized support may be a plate with wells, such as a microtitre plate. Each assay can therefore be carried out in a separate well in the plate.

Page 16, replace the first paragraph starting at line 5 with the following:

The invention also provides a peptide whose sequence is ~~represented by~~ any one of SEQ ID [[NO's]] NOS: 3, 4, 5, 6, 7, 8, 9, 10, 11 [[or]] and 12 or an analogue thereof. The invention provides a diagnostic product or panel comprising one or more of these peptides typically in the combinations discussed above. Such a product is typically a composition such as a pharmaceutical composition.

Page 16, replace the second paragraph starting at line 10 with the following:

The invention also provides a polynucleotide which is capable of expression to provide a peptide comprising the sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12; or an analogue thereof Typically the polynucleotide is DNA or RNA, and is single or double stranded. The polynucleotide therefore comprises sequence which encodes the sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12. To the 5' and 3' of this coding sequence the polynucleotide of the invention has sequence or codons which are different from the sequence or codons 5' and 3' to these sequences in the ESAT-6 gene. Therefore the polynucleotide of the invention does not comprise the sequence coding for the whole of ESAT-6 or fragments of ESAT-6, other than ~~sequence~~ sequences coding for fragments ~~represented by~~ of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 [[or]] and 12.

Page 19, replace the fourth paragraph starting at line 15 with the following:

(ii) determining whether the CD8 T cells ~~recognise~~ recognize (a) or (b) by detecting the expression of a substance by the T cells, the expression of the substance indicating that the T cells have ~~recognised~~ recognized (a) or (b), and that (a) or (b) contain a CD8 epitope or a mimic of a CD8 epitope.

Page 20, replace the third paragraph starting at line 16 with the following:

Determination of whether a T cell ~~recognises~~ recognizes the peptide is generally done by any of the methods discussed above in regard to the detection method provided by the invention.

Page 28, replace the second paragraph starting at line 13 with the following:

Two CD8 epitopes in ESAT-6 were identified. The T cells from donor NPH54, who had tuberculosis mediastinal lymphadenitis, ~~recognised~~ recognized peptides corresponding to both of these epitopes. Uncultured PBMCs isolated at the time of the diagnosis from NPH54, who has HLA-B52 and HLA-A2.01, secreted IFNγ in response to an ESAT-6 derived peptide pool for these class I alleles in an *ex vivo* ELISPOT assay. The mean number of IFNγ SFCs enumerated from 5 x 10^5PBMCs in duplicate wells was 19 for the ESAT-6 peptides compared with 2 in the control wells with no peptide. A subsequent assay tested freshly isolated PBMCs against each of the individual peptides within the responding pools; IFNγ SECs were detected in response to peptides E and G, whose sequences were congruent with the HLA-B52 and HLA-A2.01 peptide motifs, respectively. The frequency of E (1:50,000) and G (1:23,000) specific IFNγ SECs is of the same order of magnitude as SFCs for HLA-A2.01-restricted influenza matrix epitope M1 58-66 (1:14,000) (Fig 1A). Unrestimulated PBMCs from a second donor, NPH97, with tuberculosis osteomyelitis, also ~~recognised~~ recognized the G peptide. This patient also has HLA-B52 and HLA-A2.01, and the magnitude of the G specific response was similar to the response to the HLA-A2-restricted influenza matrix epitope. Single cell IFNγ release by freshly isolated T cells in these short 12-hr *ex vivo* assays, employing no stimulus other than cognate peptide, indicated that these cells are highly likely to be circulating activated effector T ~~cell~~ cells (2).

Page 30, replace the first paragraph starting at line 16 with the following:

G specific T cell lines were generated from NPH54 and NPH97 PBMCs. Depletion experiments demonstrated that-the G specific T cells are CD8 (Fig. 1B [[*B*]]). Enumeration of IFN-γ SFCs in a 12 hr ELISPOT assay for IFN-γ with cell line 3-20 from donor NPH54 was performed and is shown. After depletion of CD4 or CD8 cells, 20,000 cells were added to each of a pair of duplicate wells and peptide G was added to a final concentration of 2μM: the mean number of SFCs is shown in Fig. 1B. No SFCs were observed in the absence of peptide. CD8 cell depletion completely abrogates the response. Similar results were obtained with cell line 3-9. Similar depletion studies on E specific STCLs from donor WB, a healthy contact with HLA-A2.01, confirmed that these E specific T cells are also CD8 (Fig. 1C). After magnetic depletion of CD4 or CD8 cells, 20,000 cells were added to each of a pair of duplicate wells in a 12 hr ELISPOT assay and peptide added at 2 μM to the supernatant. The mean number of IFNγ SFCs for each pair of wells is shown in Fig 1C. CD4-depleted E specific STCLs from donor NPH43, a patient with lymphadenitis (class I HLA haplotype. HLA-A2.01, HLA-A29, HLA-B7, and HLA-B51), ~~recognised~~ recognized peptide presented through HLA-A2.01 on E-repulsed HLA-A2.01 matched heterologous BCL. Ninety eight IFNγ SFCs were enumerated in response to the E-pulsed BCL, compared with 48 IFNγ SECs for the unpulsed control BCL; the high backgrounds are probably due to alloresponse (24). Responses to E were transient and often undetectable in PBMCs from subsequent blood samples drawn later in the course of therapy.

Page 31, replace the third paragraph starting at line 27 with the following:

To show that the G-specific CD8 T cell clones were capable of ~~recognising~~ recognizing endogenously processed antigen, autologous BCL, infected with vaccinia virus recombinant for ESAT-6 (rVV-ESAT-6) or a control lacking the ESAT-6 sequence, were used to stimulate cytokine release. BCL were infected the night before with the respective recombinant viruses at a multiplicity of infection (m.o.i) of 7 plaque forming units per cell in serum free medium; after 90 min, cells were diluted up to 1 million/ml in

R10 and incubated overnight. Infected BCL (100,000) were then added to each well along with 5,000 cloned T cells. Only clones 3-1, 3-15, and 3-98 incubated with the ESAT-6 recombinant vaccinia-infected BCL secreted IFN-γ in the ELISPOT assay. 3-15 gavie in excess of 450 SFCs. The results with the other two clones, 3-1, 3-98, were so strongly positive that the spots were confluent.

Page 33, replace the first paragraph starting at line 7 with the following:

The observation that G specific T cell lines and clones ~~recognise~~ recognize target cells infected with vaccinia virus recombinant for ESAT-6 indicates that this antigen can be endogenously processed through the MHC class I antigen processing pathway, resulting in the presentation of the epitope G through HLA-B52. Because responses to the *M. tuberculosis* specific peptide ES12 were elicited from freshly isolated, unrestimulated lymphocytes in an *ex vivo* assay, CD8 T cells must have been primed through recognition of processed antigen *in vivo*. This study thus provides evidence that in humans an *M. tuberculosis* antigen is naturally processed *in vivo* through the MHC class I pathway leading to the induction of MHC class I restricted effector T cells. Further support comes from preliminary data showing that human macrophages infected with *M. tuberculosis in vitro* are ~~recognised~~ recognized by G specific HLA-B52 restricted CTL that suppress mycobacterial growth (data not shown).

Page 34, replace the second paragraph starting at line 17 with the following:

NPH 144, a patient with miliary and meningeal tuberculosis ~~recognises~~ recognizes two different epitopes. CD8+ CTL lines specific for the 15mer NLARTISEAGQAMAS (SEQ ID NO:6) are strongly cytolytic. The minimal epitope, RTISEAGQAM (SEQ ID NO:9), has been defined. T cell lines have been generated against this epitope from *M. tuberculosis*-infected individuals and these lines have been shown to be CD8 positive (by immunomagnetic depletion prior to interferon-gamma ELISPOT assay) and cytolytic (in chromium release cytotoxicity assays). The epitope is restricted through HLA-B5702, as evidenced by T cell recognition of HLA-B5702-matched, but not mismatched, peptide-pulsed B cell lines in chromium release and

interferon-gamma ELISPOT assays. CD8 T cells specific for this epitope have been detected at high frequencies directly from the peripheral blood of *M. tuberculosis*-infected individuals in ~~ex vivo~~ *ex vivo* interferon-gamma ELISPOT assays.

Page 34, replace the third paragraph starting at line 29 with the following:

A second CD8+ T cell epitope is also ~~recognised~~ recognized by NPH144. This epitope is contained within the 15mer TATELNNALQNLART (SEQ ID NO:5) and is represented by TATELNNAL (SEQ ID NO:8) ~~has been defined~~. T cell lines and clones have been generated against this 9mer peptide from *M. tuberculosis*-infected individuals and these lines have been shown to be CD8 positive (by immunomagnetic depletion prior to interferon-gamma ELISPOT assay and FACS analysis) and cytolytic (in chromium release cytotoxicity assays). The epitope is restricted through HLA-B3503, as evidenced by T cell recognition of HLA-B3503-matched, but not mismatched, peptide-pulsed B cell lines in chromium release and interferon-gamma ELISPOT assays. CD8 T cells specific for this epitope have been detected at high frequencies directly from the peripheral blood of *M. tuberculosis*-infected individuals in ~~ex vivo~~ *ex vivo* interferon-gamma ELISPOT assays and by peptide-MHC tetramers.

Page 35, replace the second paragraph starting line 14 with the following:

In an *ex-vivo* 9-hr ELISPOT assay with freshly isolated PBL from NPH 144 responses to peptide ES15 (TATELNNALQNLART, SEQ ID NO:5) and ES13 (NLARTISEAGQAMAS, SEQ ID NO:6) are found within 9-hrs of antigen contact (Figures 3 and 4). This *ex-vivo* response is abrogated by CD8 depletion with Dynabeads. The frequency of circulating T cells specific for ES15 in NPH144 ranges from 1/500 -1/3,000. The mild diminution in the response with CD4 depletion indicated that peptide ES15 may also be a target of IFN-γ secreting CD4+ T cells in NPH144, as it is in many other tuberculosis patients and contacts.

Page 36, replace the first paragraph starting line 2 with the following:

Within the 15mer peptide SGSEAYQGVQQKWDA (SEQ ID NO:7), the minimal epitope, AYQGVQQKW (SEQ ID NO:10) has been defined. T cell lines have been generated against this epitope from ~~M. tuberculosis~~ *M. tuberculosis*-infected individuals and these lines have been shown to be CD8 positive (by immunomagnetic depletion prior to interferon-gamma ELISPOT assay) and cytolytic (in chromium release cytotoxicity assays). The epitope is restricted through HLA-A24, as evidenced by T cell recognition of HLA-A24-matched, but not mismatched, peptide-pulsed B cell lines in chromium release and interferon-gamma ELISPOT assays. CD8 T cells specific for this epitope have been detected at high frequencies directly from the peripheral blood of ~~M. tuberculosis~~ *M. tuberculosis*-infected individuals in ~~ex vivo~~ *ex vivo* interferon-gamma ELISPOT assays. Additionally, a second CD8 epitope exists ~~with~~ within the 15mer SGSEAYQGVQQKWDA (SEQ ID NO:7). This epitope is restricted through HLA-B44, as evidenced by T cell recognition of HLA-B44-matched, but not mismatched, peptide-pulsed B cell lines in interferon-gamma ELISPOT assays. The identity of the precise minimal epitope is under investigation and, on the basis of the HLA-B44 peptide motif, is very likely to be either SEAYQGVQQ (SEQ ID NO:11) or SEAYQGVQQK (SEQ ID NO:12).

Page 36, replace the second paragraph starting line 20 with the following:

We have identified 8 CD8 T cell epitopes in ESAT-6. These epitopes are restricted through HLA-B52 (for LQNLARTI, SEQ ID NO:2), A2 (for AMASTEGNV, SEQ ID NO:1), A68.02 (for NVTSIHSLL, SEQ ID NO:3), B3503 (for TATELNNAL, SEQ ID NO:8), B5702 (for RTISEAGQAM, SEQ ID NO:9), A24 (for AYQGVQQKW, SEQ ID NO:10) and B44 (for the second epitope present in SGSEAYQGVQQKWDA, SEQ ID NO:7). ESAT-6, only 95 amino acids in length, is thus extraordinarily rich in CD8+ CTL epitopes. Some of these epitopes moreover overlap with certain human CD4+ epitopes (e.g. peptide ES15).

Page 36, replace the fourth paragraph starting line 30 with the following:

We have shown that ESAT-6-specific CD8+ CTL can affect an unusual delayed suppression of the growth of *M. tuberculosis in vitro* but the role of these CD8+ CTL in humans *in vivo* is not known. One of our subjects, NPH130, a healthy household contact, has a high frequency (1/1,000 PBL) of CD8+ CTL specific for the HLA-A68.02 restricted epitope NVTSIHSLL (SEQ ID NO:3), as demonstrated by *ex vivo* ELISPOT (Figure 5). The presence of such a high frequency of *M. tuberculosis* specific CD8+ CTLs in an exposed but healthy individual with a clinically undetectable bacillary load raises the possibility that these T cells may, in some individuals, be associated with containment of *M. tuberculosis in vivo*.